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Original article

Nymphs of the genus *Amblyomma* (Acari: Ixodidae) of Brazil: descriptions, redescriptions, and identification key

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ABSTRACT

Together with the larval stage, the nymphal stage of ticks of the genus *Amblyomma* are the most aggressive ticks for humans entering areas inhabited by wildlife and some domestic animals in Brazil. However, due to the absence of morphological descriptions of the nymphal stage of most Brazilian *Amblyomma* species, plus the lack of an identification key, little or nothing is known about the life history of *Amblyomma* spp. nymphs in the country. In the present study, morphological description of the nymphal stage, illustrating important external characters through scanning electron microscopy, is provided for nymphs of 15 *Amblyomma* species that occur in Brazil, for which the nymphal stage had never been described: *A. aureolatum*, *A. auricularium*, *A. calcaratum*, *A. coelebs*, *A. fuscum*, *A. humerale*, *A. incisum*, *A. latepunctatum*, *A. naponense*, *A. nodosum*, *A. ovale*, *A. pacae*, *A. pseudoconcolor*, *A. scalpturatum*, *A. varium*. In addition, the nymphal stage of 12 *Amblyomma* species, which had been previously described, are redescribed: *A. brasiliense*, *A. cajennense*, *A. dissimile*, *A. dubitatum*, *A. longirostre*, *A. oblongoguttatum*, *A. parkeri*, *A. parvum*, *A. romitii*, *A. rotundatum*, *A. tigrinum*, *A. triste*. The descriptions and redescriptions totalized 27 species. Only 2 species (*A. geayi*, *A. goeldii*) out of the 29 *Amblyomma* species established in Brazil are not included in the present study. A dichotomous identification key is included to support taxonomic identification of the nymphal stage of 27 *Amblyomma* species established in Brazil.

Introduction

The genus *Amblyomma* (Acari: Ixodidae) is currently represented by 130 valid tick species (Nava et al., 2009a, 2009b), 29 of which (22.3%) are established in Brazil (Dantas-Torres et al., 2009). While the adult stage of these 29 species has been morphologically well described, resulting in good identification keys, the same cannot be said for the immature stages (Guglielmone et al., 2003; Onofrio et al., 2006). The nymphal stage of 17 *Amblyomma* species from Brazil remains undescribed. This scenario has precluded the construction of identification keys. Consequently, taxonomic identification to species level of field-collected *Amblyomma* nymphs has been a laborious and very difficult task, sometimes impossible. For example, species identification of field-collected *Amblyomma* nymphs in recent studies in Brazil were possible only because these nymphs were reared to the adult stage in the laboratory or because molecular methods were applied, such as DNA sequencing of molecular markers previously determined for each species through the adult stage (Labruna et al., 2007; Ogrzewalska et al., 2009).

Herein, we describe the nymphal stage of 15 *Amblyomma* species for the first time and redescribe the nymphal stage of another 12 *Amblyomma* species from Brazil. In addition to providing scanning electron micrographs of the nymphs, we also include an identification key for the 27 *Amblyomma* species evaluated in the present study.

Materials and methods

During 2000–2009, tick colonies of 23 *Amblyomma* species from Brazil were maintained in the laboratory of the Faculty of Veterinary Medicine of the University of São Paulo at different periods for different purposes. Unfed nymphs, 15–30 days old, of the first laboratory generation (F_1) of each species were killed in hot water (70–80 °C) and immediately preserved in 70% alcohol until further processing for description or redescription. In addition, alcohol-preserved F_1 unfed nymphs from laboratory colonies of 3 other *Amblyomma* species were kindly provided by colleagues from other institutions, as stated in the Acknowledgments. All these 26 tick colonies started with field-collected ticks as stated in Table 1. Species identification of the tick colonies was undertaken during the adult stage (F_0) using specific identification keys and corresponding morphological redescriptions available in the current literature (Robinson, 1926; Aragão and Fonseca, 1961; Jones et

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Table 1
Origin of tick colonies used to obtain F_1 nymphs described or redescribed in the present study.

Tick species	Laboratory colonies					
	Origin				Nymphs used	
	Stage	Host	Locality	Date	Accession no.	*N*
Amblyomma aureolatum	Female	*Canis familiaris*	Bom Jesus dos Perdões, SP, Brazil	04 October 2007	CNC-1458	10
A. auricularium	Female	*Dasypus novemcinctus*	Mossoró, RN, Brazil	12 December 2007	CNC-1459	10
A. calcaratum	Female	*Myrmecophaga tridactyla*	Sorocaba, SP, Brazil.	19 February 2008	CNC-1460	10
A. coelebs	Female	*Tapirus terrestris*	Teodoro Sampaio, SP, Brazil	04 September 2007	CNC-1461	10
A. fuscum	Larvae, nymphs	*Didelphis aurita*	Guarujá, SP, Brazil	26 May 2008	CNC-1462	10
A. humerale	Female	*Geochelone denticulata*	Linhares, ES, Brazil	18 September 2006	CNC-1463	10
A. incisum	Males, females	Free-living	Ribeirão Grande, SP, Brazil	11 May 2005	CNC-1464	10
A. latepunctatum	Males, females	Free-living	Governador Jorge Teixeira, RO, Brazil	31 January 2008	CNC-1465	10
A. naponense	Female	*Tayassu pecari*	Teodoro Sampaio, SP, Brazil	29 November 2004	CNC-1466	10
A. nodosum	Female	*M. tridactyla*	Presidente Epitácio, SP, Brazil	23 March 2001	CNC-1467	10
A. ovale	Female	*C. familiaris*	Monte Negro, RO, Brazil	23 November 2001	CNC-1468	10
A. pacae	Female	*Cuniculus paca*	Monte Negro, RO, Brazil	23 February 2002	CNC-1469	10
A. pseudoconcolor	Female	*Euphractus sexcinctus*	Água Morta, Salta, Argentina	25 July 2008	CNC-1470	6
A. scalpturatum	Males, females	Free-living	Governador Jorge Teixeira, RO, Brazil	31 January 2008	CNC-1471	10
A. varium	Female	*Bradypus variegatus*	Sorocaba, SP, Brazil	20 April 2000	CNC-0291	10
A. brasiliense	Female	*Pecari tajacu*	Teodoro Sampaio, SP, Brazil	06 March 2007	CNC-1472	10
A. cajennense	Female	*Equus caballus*	Pirassununga, SP, Brazil	01 March 2007	CNC-1473	10
A. dissimile	Female	*Iguana iguana*	Cuiabá, MT, Brazil	04 October 2007	CNC-1474	10
A. dubitatum	Female	*Hydrochoerus hydrochaeris*	Miracatu, SP, Brazil	04 December 2007	CNC-1475	10
A. longirostre	Female	*Sphiggurus villosus*	São Paulo, SP, Brazil	25 June 2008	CNC-1476	10
A. oblongoguttatum	Female	*C. familiaris*	Monte Negro, RO, Brazil	04 October 2007	CNC-1477	10
A. parvum	Female	*C. familiaris*	Barão de Melgaço, MT, Brazil	27 September 2002	CNC-1478	10
A. romitii	Female	*H. hydrochaeris*	Rurópolis, PA, Brazil	17 April 2009	CNC-1479	10
A. rotundatum	Female	*Boa constrictor*	Porto Velho, RO, Brazil	08 February 2008	CNC-1480	10
A. tigrinum	Female	*C. familiaris*	Herval, RS, Brazil	03 July 2002	CNC-1481	10
A. triste	Female	*Blastocerus dichotomus*	Bataguassu, MS, Brazil	10 January 2001	CNC-1482	10
A. parkeri	Nymph[a]	*Coendou* sp.	Cotia, SP, Brazil	15 March 1933	IBSP-4458	1

CNC: Tick collection "Coleção Nacional de Carrapatos da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo". IBSP: Tick collection "Coleção Acarológica do Instituto Butantan, São Paulo". *N*: No. of specimens used for description or redescription, deposited in the designated tick collection.

[a] Only a single engorged nymph was available for redescription.

al., 1972; Estrada-Peña et al., 2002; Barros-Battesti et al., 2005, 2007; Labruna et al., 2005; Onofrio et al., 2006, 2008). In the case of *A. parkeri* Fonseca and Aragão, 1952, no laboratory colony was available; therefore we used a single engorged nymphal specimen for redescription that had been described by Fonseca and Aragão (1952) (Table 1).

The nymphal stage of the 27 *Amblyomma* species listed in Table 1 were described or redescribed based on optical microscopy, following previous authors who described *Amblyomma* nymphs from the New World (Cooley and Kohls, 1944; Guglielmone et al., 1990; Estrada-Peña et al., 1993, 2002; Nava et al., 2009b). For this purpose, 10 specimens of each species (except for *A. pseudoconcolor* Aragão, 1908, and *A. parkeri*, for which only 6 and 1 specimen, respectively, were available) were measured using the Image-Pro Plus 5.1 program for analysis of images and morphometry, fitted to an Olympus SZX stereoscope microscope. In the descriptions/redescriptions that follow, all measurements are given in millimeters; first the mean ± standard deviation, followed by the range in parentheses. With the exception of *A. parkeri*, representative specimens of all *Amblyomma* species were prepared for scanning electron microscopy (SEM) following techniques described by Corwin et al. (1979).

Finally, we constructed a dichotomous identification key based on morphological differences and similarities of the nymphal stage of the 27 *Amblyomma* species evaluated in the present study.

Results

Descriptions

Amblyomma aureolatum (Pallas, 1772) (Fig. 1)

Idiosoma. Length from apices of scapula to posterior body margin 1.287 ± 0.072 (1.104–1.365), maximum breadth 0.907 ± 0.045 (0.794–0.956), outline oval, longilinear, with 11 festoons without tubercles. *Scutum*. Length 0.660 ± 0.038 (0.568–0.707), breadth 0.731 ± 0.022 (0.679–0.766), breadth/length ratio 1.109 ± 0.035 (1.081–1.194), inornate, surface extensively shagreened (rugose); few punctations, larger and deeper laterally. Eyes not orbited at lateral scutal angles at the level of scutal midlength. Cervical grooves reaching the scutal midlength, deeper at the anterior half. Spiracular plate triangular with rounded angles, with a discrete dorsal prolongation; length 0.053 ± 0.002 (0.050–0.057), breadth 0.026 ± 0.002 (0.021–0.028). *Gnathosoma (capitulum).* Length from palpal apices to posterior margin 0.306 ± 0.030 (0.236–0.337), breadth 0.297 ± 0.012 (0.273–0.316). Basis capituli triangular, posterior margin slightly concave, without cornua; posterior margin convex ventrally, without auriculae, lateral margin projected laterally. Palpi length 0.230 ± 0.018 (0.184–0.246), article I without ventral prolongation, article II 0.152 ± 0.010 (0.127–0.167) long, article III 0.055 ± 0.006 (0.045–0.064) long. Hypostome rounded apically; length 0.212 ± 0.026 (0.169–0.267); length of toothed portion 0.119 ± 0.011 (0.098–0.136); dentition 2/2 with 7–8 teeth per row. *Legs*. Coxa I with 2 stout spurs close to each other, the external longer; coxae II–IV with a small triangular spur that gradually decreases in size from II to IV. Trochanters without spur; tarsus I 0.364 ± 0.023 (0.316–0.396) long, 0.122 ± 0.013 (0.105–0.149) broad; tarsus IV 0.286 ± 0.022 (0.253–0.319) long, 0.092 ± 0.017 (0.073–0.125) broad.

Amblyomma auricularium (Conil, 1878) (Fig. 2)

Idiosoma. Length from apices of scapula to posterior body margin 1.256 ± 0.076 (1.107–1.333), maximum breadth 0.951 ± 0.057 (0.816–1.007); outline oval, slightly longilinear, with 11 festoons without tubercles. *Scutum*. Length 0.629 ± 0.043 (0.525–0.664), breadth 0.767 ± 0.039 (0.679–0.812), breadth/length ratio 1.221 ± 0.029 (1.184–1.294), inornate; few medium punctations